

CLAIMS

1. A method for investigating at least one tissue layer in living animals using a microscope, comprises the following steps:
 - focusing the microscope onto the tissue layer being investigated,
 - generating signals that correspond to the positional changes of the tissue layer brought about by movements of the animal or by movements of the animal's organs, or that at least indicate the beginning of a positional change;
 - acquiring images or/and performing optical measurements even during the positional changes, and
 - storing the signals, together with the corresponding images or/and the results of the measurements, for later evaluation, in which the images or measurements made when the layer being investigated is in focus are identified on the basis of the signals, or
 - processing the signals in such a way that the positional changes are compensated for in real time and the tissue layer being investigated is thereby always in focus.
2. The method as defined in Claim 1, wherein the signals corresponding to the positional changes of the tissue layer are generated by optical scanning of the tissue layer or of other parts of the animal triggering the movement of the tissue layer.
3. The method as defined in Claim 2, wherein the optical scanning is performed by scanning with a laser and by acquisition of images and corresponding image processing.

4. The method as defined in Claim 1, wherein the signals corresponding to the positional changes of the tissue layer are generated by the animal's heartbeat.
5. The method as defined in Claim 4, wherein the heartbeat is measured by means of electrodes or by way of the animal's pulse.
6. The method as defined in Claim 1, wherein the positional changes of the tissue layer are measured in advance for calibration.
7. The method as defined in Claim 1, wherein the compensation for the positional changes is accomplished in real time by way of a focusing device.
8. The method as defined in Claim 1, wherein the beginning of the positional changes is stimulated electrically, in particular by way of a cardiac pacemaker.
9. A method for investigating layers of tissues in living animals using a microscope, comprises the following steps:
 - focusing the microscope onto the tissue layer being investigated,
 - stimulating the animal in order to cause defined organ movements and thus defined positional changes of the tissue layer;
 - acquiring images or/and performing optical measurements even during the positional changes, and
 - storing the images or measurement results acquired during or after the stimulation for later evaluation, or

compensating for the positional changes of the tissue layer as a function of the stimulation.

10. The method as defined in Claim 9, wherein the stimulation of the animal is accomplished at the heart or at suitable nerve points.
11. The method as defined in Claim 10, wherein the stimulation is accomplished by way of a cardiac pacemaker, the latter's cycle frequency and amplitude being selectively adjusted.
12. The method as defined in Claim 11, wherein the microscope and the amplitude of the cardiac pacemaker are set in such a way that the positional changes of the tissue layer lie substantially within the depth of field of the microscope.
13. The method as defined in Claim 9, wherein acquisition of the images, or performance of the measurement, or compensation for the positional changes of the tissue layer is accomplished after an adjustable time delay following the beginning of the positional change.
14. An apparatus for investigating at least one tissue layers in living animals comprising:
 - a microscope,
 - a focusing device for focusing the microscope onto a tissue layer;
 - a movement measuring means for direct or indirect sensing of positional changes or at least of the beginning of positional changes of the tissue layer that are brought

about by movements of the animal or of the animal's organs, the movement measuring means generating corresponding signals;
a camera for acquiring images and a measuring device for performing optical measurement even during the positional changes, and
a memory for storing the signals, together with the associated images of the camera or together with the measurement results of the measuring device, for later evaluation.

15 The apparatus as defined in Claim 14, wherein a control unit provided for processing the signals in such a way that the positional changes of the tissue layer are compensated for in real time.

16. The apparatus as defined in Claim 14, wherein the movement measuring means senses the positional changes by optical measurement, image acquisition, mechanical measurement, or electrical measurement at electrodes on the animal.

17. An apparatus for investigating at least one tissue layer in living animals, comprising :
a microscope,
a focusing device for focusing the microscope onto a tissue layer;
a stimulation means for causing defined organ movements and therefore defined positional changes of the tissue layer;
a camera for acquiring images and a measuring device for performing optical measurement even during the positional changes, and
a memory for storing the images acquired during or after the stimulation, or for storing measurement results of the measurement device, for later evaluation.

18. The apparatus as defined in Claim 17, wherein a control unit is provided for compensating for the positional changes of the tissue layer as a function of the stimulation.
19. The apparatus as defined in Claim 17, wherein the stimulation means is a cardiac pacemaker.
20. The apparatus as defined in Claim 17, wherein a means for time delay is provided, with which the beginning of the image acquisitions or measurements on the tissue layer are shifted in time with respect to the beginning of the positional changes of the tissue layer or the beginning of the stimulation.
21. The apparatus as defined in Claim 17 wherein the microscope is a laser scanning microscope.
22. The apparatus as defined in Claim 17, wherein the measurement device is a photometer, a polarimeter, or a fluorescence device.
23. The apparatus as defined in Claim 17, wherein the focusing device is a piezoelectric focusing device or a focusing nosepiece.